

AMENDMENTS

To the claims:

Please amend the claims as indicated hereafter.

1. (Currently Amended) A method of isolating islets from a pancreas, comprising the steps of:

introducing a pancreas or portion of a pancreas to an islet processing solution;

circulating an islet processing solution containing a tissue dissociating compound

around and through the pancreatic tissue;

controlling one or more process control variables of ~~an~~ the islet processing

solution during islet isolation with a process controller, wherein one of the

one or more process control variables is ~~the~~ a process temperature (T);

controlling the process temperature (T) of the islet processing solution during

islet isolation between 4.0 and 44.0 degrees Celsius; ~~and~~

~~and~~ separating one or more islets from the pancreatic tissue pancreas; ~~and~~

collecting the separated islets.

2. (Previously Presented) The method of claim 1, wherein the process controller is a PID (proportional, integral, derivative) controller.

3. (Previously Presented) The method of claim 1, wherein the process controller is a microprocessor temperature controller.

4. (Previously Presented) The method of claim 1, wherein the process controller is a microprocessor controller.

5. (Previously Presented) The method of claim 1, wherein the process controller is a microprocessor computer.

6. (Previously Presented) The method of claim 1, wherein the process controller is a variable resistance transformer.

7. (Previously Presented) The method of claim 1, wherein the process temperature is adjusted by an electrical resistance element in thermal communication with the islet processing solution.

8. (Previously Presented) The method of claim 1, wherein the process temperature is adjusted by steam placed in thermal communication with the islet processing solution.

9. (Previously Presented) The method of claim 1, wherein the process temperature is adjusted by a recirculating fluid bath in thermal communication with the islet processing solution.

10. (Previously Presented) The method of claim 1, wherein the process temperature is adjusted by the temperature of the ambient surrounding in thermal communication with the islet processing solution.

11. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process percent hydrogen ion (pH) concentration and the pH is controlled by a microprocessor pH controller between pH 6.00 and pH 8.00.

12. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process percent hydrogen ion (pH) concentration and the pH is controlled by a microprocessor controller between pH 6.00 and pH 8.00.

13. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process percent hydrogen ion (pH) concentration and the pH is controlled by a microprocessor computer between pH 6.00 and pH 8.00.

14. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process percent hydrogen ion (pH) concentration and the pH is controlled by the addition of an acid or base to the islet processing solution.

15. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process flowrate (F) and the flowrate is controlled by a microprocessor flow controller between 10.0 milliliters per minute (10.0 ml/min) and 4000.0 milliliters per minute (4000.0 ml/min).

16. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process flowrate (F) and the flowrate is controlled by a microprocessor controller between 10.0 milliliters per minute (10.0 ml/min) and 4000.0 milliliters per minute (4000.0 ml/min).

17. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process flowrate (F) and the flowrate is controlled by a microprocessor computer between 10.0 milliliters per minute (10.0 ml/min) and 4000.0 milliliters per minute (4000.0 ml/min).

18. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process dissolved oxygen (DO) concentration and the DO concentration is controlled by a microprocessor DO controller between 0.000000001 milligrams per milliliter (0.000000001mg/ml) DO and 10.0 milligrams per milliliter (10.0 mg/ml) DO.

19. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process dissolved oxygen (DO) concentration and the DO concentration is controlled by a microprocessor controller between 0.000000001 milligrams per milliliter (0.000000001mg/ml) DO and 10.0 milligrams per milliliter (10.0 mg/ml) DO.

20. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process dissolved oxygen (DO) concentration and the DO concentration is controlled by a microprocessor computer between 0.000000001 milligrams per milliliter (0.000000001mg/ml) DO and 10.0 milligrams per milliliter (10.0 mg/ml) DO.

21. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process dissolved oxygen (DO) concentration and the DO concentration is controlled by sparging the islet processing solution with ~~an~~ at least one inert gas chosen from helium, neon, argon, krypton, or xenon.

22. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process dissolved nitric oxide (NO) concentration and the NO concentration is controlled by a microprocessor NO controller between 0.000000000000001 moles per liter (0.01 picomoles/liter) NO and 1.0 mole per liter (1.0 mol/liter) NO.

23. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process dissolved nitric oxide (NO) concentration and the NO concentration is controlled by a microprocessor controller between 0.000000000000001 moles per liter (0.01 picomoles/liter) NO and 1.0 mole per liter (1.0 mol/liter) NO.

24. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process dissolved nitric oxide (NO) concentration and the NO concentration is controlled by a microprocessor computer between 0.000000000000001 moles per liter (0.01 picomoles/liter) NO and 1.0 mole per liter (1.0 mol/liter) NO.

25. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process dissolved nitric oxide (NO) concentration and the NO concentration is controlled by sparging the islet processing solution with ~~an~~ at least one inert gas chosen from helium, neon, argon, krypton, or xenon.

26. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process endotoxin (E) concentration and the endotoxin concentration is controlled by a microprocessor E controller between 0.000000001 endotoxin units (EU) per milligram (1.0 nanoEU/mg) and 100.0 endotoxin units per milligram (100.0 EU/mg).

27. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process endotoxin (E) concentration and the endotoxin concentration is controlled by a microprocessor controller between 0.000000001 endotoxin units (EU) per milligram (1.0 nanoEU/mg) and 100.0 endotoxin units per milligram (100.0 EU/mg).

28. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process endotoxin (E) concentration and the endotoxin concentration is controlled by a microprocessor computer between 0.000000001 endotoxin units (EU) per milligram (1.0 nanoEU/mg) and 100.0 endotoxin units per milligram (100.0 EU/mg).

29. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process endotoxin (E) concentration and the endotoxin concentration is controlled by the addition of an endotoxin neutralizing protein (ENP) to the islet processing solution.

30. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process endotoxin neutralizing protein (ENP) concentration and the ENP concentration is controlled by a microprocessor controller between 0.000000000000001 moles per liter (0.01 picomoles/liter) ENP and 1.0 moles per liter (1.0 mol/liter) ENP.

31. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process proteolytic enzyme [PE] activity (~~measured by the metalloendoproteinase [collagenase] concentration~~) and the proteolytic enzyme activity is controlled by the addition of one or more antibiotics to the islet processing solution chosen from tetracycline, minocycline, or doxycycline.

32. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process proteolytic enzyme [PE] activity (~~measured by the metalloendoproteinase [collagenase] concentration~~) and the proteolytic enzyme activity is controlled by the addition of one or more chelators of divalent cations to the islet processing solution chosen from citrate, EDTA, or EGTA.

33. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process proteolytic enzyme [PE] activity (~~measured by the metalloendoproteinase [collagenase] concentration~~) and the proteolytic enzyme activity is controlled by the addition of one or more amino acids to the islet processing solution chosen from cysteine or cystine.

34. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process proteolytic enzyme [PE] activity and the proteolytic enzyme is controlled by a microprocessor controller between 0.00000000000001 moles per liter (0.01 picomoles/liter) and 1.0 moles per liter (1.0 mol/liter).

35. (Canceled)

36. (Currently Amended) The method of claim 1, wherein ~~the~~ a second process control variable is ~~the~~ a process antibiotic (A) concentration and the antibiotic concentration is controlled by a microprocessor controller between 0.00000000000001 moles per liter (0.01 picomoles/liter) A and 1.0 mole per liter (1.0 mol/liter) A.

37. (Currently Amended) The method of claim 1, wherein ~~the~~-a second process control variable is ~~the~~-a process nitric oxide synthase (NOS) concentration and the nitric oxide synthase concentration is controlled by the addition to the islet processing solution of one or more derivatives of L-arginine ~~to the islet processing solution~~ chosen from aminoguanidine, N, N'-diaminoguanidine, methylguanidine, or 1, 1-dimethylguanidine.

38. (Currently Amended) The method of claim 1, wherein ~~the~~-a second process control variable is ~~the~~-a process nitric oxide synthase (NOS) concentration and the nitric oxide synthase concentration is controlled by the addition of 2,4-diamino-6-hydroxy-pyrimidine to the islet processing solution.

39. (Currently Amended) The method of claim 1, wherein ~~the~~-a second process control variable is ~~the~~-an islet processing solution pressure (P) and the pressure is between 1.0 pound per square inch gauge (psig) pressure and 150.0 pounds per square inch gauge (psig) pressure.

40. (Currently Amended) The method of claim 1, wherein ~~the~~-a second process control variable is ~~the~~-a process carbon monoxide (CO) concentration and the carbon monoxide concentration is controlled by sparging the islet processing solution with carbon monoxide.

41. (Previously Presented) The method of claim 1, wherein the pancreas is a human pancreas.

42. (Previously Presented) The method of claim 1, wherein the pancreas is a transgenic porcine pancreas.

43. (Previously Presented) The method of claim 1, wherein the pancreas is a non-transgenic porcine pancreas.

44. (Previously Presented) The method of claim 1, wherein the pancreas is a transgenic mammalian pancreas.

45. (Previously Presented) The method of claim 1, wherein the pancreas is a non-transgenic mammalian pancreas.

46. (Previously Presented) The method of claim 1, wherein the pancreas is a transgenic fish pancreas.

47-60. (Canceled)

61. (Currently Amended) A method of isolating islets from a pancreas, comprising the steps of:

introducing a pancreas or portion of a pancreas to an islet processing solution;

circulating an islet processing solution containing a tissue dissociating compound around and through the pancreatic tissue;

controlling one or more process control variables of ~~an~~ the islet processing solution during islet isolation in a predetermined manner, the one or more process control variables comprises at least one chosen from the group comprising: the following: temperature, pH, flowrate, dissolved oxygen concentration, dissolved nitric oxide concentration, nitric oxide synthase concentration, endotoxin concentration, endotoxin neutralizing protein concentration, antibiotic concentration, amino acid concentration, dextran concentration, heparin concentration, or proteolytic enzyme activity; ~~and~~ separating one or more islets from the pancreatic tissue ~~a pancreas~~ while the one or more process control variables is controlled; and collecting the separated islets.

62. (Currently Amended) The method claim 61, wherein a second process control variable is ~~the~~ a process proteolytic enzyme [PE] activity and the proteolytic enzyme activity is controlled by ~~the~~ an addition of antibiotics to the islet processing solution.

63. (Currently Amended) The method claim 61, wherein a second process control variable is ~~the~~ a process proteolytic enzyme [PE] activity and the proteolytic enzyme activity is controlled by ~~the~~ an addition of chelators of divalent cations to the islet processing solution.

64. (Currently Amended) The method of claim 61, wherein a second process control variable is ~~the~~ a process proteolytic enzyme [PE] activity and the proteolytic enzyme activity is controlled by ~~the~~ an addition of amino acids to the islet processing solution.

65. (Currently Amended) The method of claim 61, wherein a second process control variable is ~~the~~ a process dissolved nitric oxide (NO) concentration and the NO concentration is controlled or inhibited by ~~the~~ an addition to the islet processing solution of one or more ~~of~~ derivatives of L-arginine ~~to the islet processing solution~~ chosen from aminoguanidine, N, N'-diaminoguanidine, methylguanidine, or 1, 1-dimethylguanidine.

66. (Currently Amended) The method of claim 61, wherein a second process control variable is ~~the~~ a process dissolved nitric oxide (NO) concentration and the NO concentration is controlled or inhibited by ~~the~~ an addition of 2,4-diamino-6-hydroxy-pyrimidine to the islet processing solution.

67. (Currently Amended) The method of claim 61, wherein a second process control variable is ~~the~~ a process dissolved nitric oxide (NO) concentration and the NO concentration is controlled or inhibited by ~~the~~ an addition of amino acids to the islet processing solution.

68. (Currently Amended) The method of claim 61, wherein a second process control variable is ~~the~~ a process dissolved nitric oxide (NO) concentration and the NO concentration is controlled or inhibited by ~~the~~ an addition of one or more of the following to the islet processing solution chosen from dextran or heparin.

69. (Currently Amended) The method of claim 61, wherein a second process control variable is ~~the~~ process dissolved nitric oxide (NO) concentration and the NO concentration is controlled or inhibited by ~~the~~ an addition one or more antibiotics to the islet processing solution chosen from tetracycline, minocycline, or doxycycline.

70. (Currently Amended) The method of claim 61, wherein a second process control variable is ~~the~~ a process nitric oxide synthase (NOS) concentration and the NOS concentration is controlled or inhibited by ~~the~~ an addition of one or more antibiotics to the islet processing solution chosen from tetracycline, minocycline, or doxycycline.

71. (Previously Presented) The method of claim 61, wherein apoptosis of beta cells is inhibited during and after islet isolation by controlling the endotoxin concentration in the islet processing solution.

72. (Previously Presented) The method of claim 61, wherein apoptosis of beta cells is inhibited during and after islet isolation by controlling the nitric oxide concentration in the islet processing solution.